Myrmicine Trail Pheromones: Specificity, Source and Significance

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Abstract: The poison gland secretion is the source of the trail pheromones in the myrmicine genera *Myrmica, Manica, Pogonomyrmex*, and *Veromessor*. Transposition studies demonstrate that poison gland products of *Myrmica, Manica*, and *Pogonomyrmex* species lack intra- and intergeneric specificity. The unpredictable lack of trail specificity identified with myrmicine venoms is discussed in terms of common trace natural products which may be utilized as trail pheromones by species in unrelated taxa. The persistence of chemical trails is discussed as a function of the foraging strategies employed by myrmicine species.

INTRODUCTION

The sources of trail pheromones in the Formicidae are quite protean, especially in the large subfamily Myrmicinae. Releasers of trail-following behavior have been localized in the poison gland (Moser and Blum, 1963), Dufour's gland (Wilson, 1959) and metathoracic tibial glands (Fletcher and Brand, 1968) of a wide range of myrmicine species, which clearly emphasizes the polyphyletic origins of trail following in this subfamily. Indeed, with the exception of some ponerine species which generate trails with poison gland secretions (Fletcher, 1971), species in formicid subfamilies other than the Myrmicinae are not known to utilize the above named organs for producing these chemical releasers.

The variability in the glandular sources of trail following in the Myrmicinae is exceeded by the variability in specificity of the products synthesized in these social organs. In some cases constituents in the poison gland secretions release trail following in different species in the same genus (Blum, 1966), but in other cases these secretions may be completely species specific when evaluated among members of one genus (Blum and Ross, 1965). Furthermore, the natural product complex in the venoms of some myrmicines can release strong trail following in species in completely unrelated genera when assayed by an artificial trail technique (Blum and Ross, 1965; Blum and Portocarrero, 1966). On the other hand, it has not been ascertained whether these singular examples of nonspecificity reflect the utilization of the same pheromone by unrelated species, or whether different poison gland secretions are enriched with common constituents, some of which may serve as trail pheromones for unrelated species.

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TABLE 1. Percentage of *Myrmica* workers responding to artificial trails prepared from poison gland extracts

Source species	Test Species						
	americana	*brevinodis*	*brevispinosa*	*emeryana*	*fracticornis*	*monticola*	*rubra*
americana	90	70	—	65	90	90	90
brevinodis	80	90	—	50	80	85	55
brevispinosa	85	50	90	65	80	55	85
emeryana	90	80	80	95	60	80	60
fracticornis	40	60	—	90	90	75	—
monticola	10	0	—	0	0	15	0
rubra	70	90	—	10	75	0	90

The present investigation was undertaken in order to determine the source and specificity of the trail pheromones of myrmicine species in a wide range of genera. The results again clearly emphasize that these pheromonally-rich secretions have a remarkable lack of intra- or intergeneric specificity.

METHODS

Workers of the following myrmicine species were utilized for studies of either the source or specificity of trail pheromones: *Aphaenogaster fulva* Roger, *Myrmica americana* Weber, *M. brevinodis* Emery, *M. brevispinosa* Wheeler, *M. emeryana* Forel, *M. monticola* Wheeler, *M. fracticornis* Emery, *M. rubra* (L.), *Manica bradleyi* (Wheeler), *M. hunteri* (Wheeler), *M. mutica* (Emery), *Pogonomyrmex badius* (Latreille), *P. barbatus* (F. Smith), *Novomessor cockerelli* (E. André), *Veromessor pergandei* (Mayr), *Chelaner antarcticum* (Wheeler), *Pheidole dentata* Mayr, *Crematogaster lineolata* (Say), *Monomorium minimum* (Buckley), *Solenopsis invicta* Buren, and *Trachymyrmex septentrionalis* (McCook).

The presence of trail pheromones was examined by preparing methylene chloride extracts of poison glands, Dufour's glands, and hind guts. Four organs were crushed in 2 ml of solvent and 0.2 ml of this extract was applied to a circular trail 15 cm in diameter. Groups of ten workers were subsequently introduced into the center of the circle and if a worker traveled around the entire circumference after encountering it, a positive response was recorded (Moser and Blum, 1963).

Specificity studies were undertaken by using the same technique. Six replicates, consisting of ten workers each, were employed for each test species.

RESULTS

No evidence of trail following could be demonstrated when workers of *Aphaenogaster fulva* and *Novomessor cockerelli* were exposed to circular trails treated with extracts of their own sting-associated glands or hind guts. On the

Table 2. Percentage of myrmicine workers responding to artificial trails prepared from poison gland extracts

Source species	Test Species					
	Myrmica americana	*Myrmica brevinodis*	*Manica bradleyi*	*Manica hunteri*	*Manica mutica*	*Pogonomyrmex badius*
A. fulva	—	0	0	—	—	—
Manica bradleyi	—	80	80	—	—	—
M. hunteri	90	—	—	95	—	40
M. mutica	90	—	—	90	65	—
Myrmica americana	90	70	—	—	—	—
M. brevinodis	80	90	—	90	90	70
M. brevispinosa	85	50	—	95	90	—
M. emeryana	90	80	—	95	85	—
M. fracticornis	40	60	—	90	90	—
M. monticola	10	0	—	85	95	—
M. rubra	70	90	65	95	95	0
N. cockerelli	—	0	0	—	—	0
P. badius	95	—	55	90	60	50
P. barbatus	—	90	50	—	—	0
V. pergandei	—	0	0	—	—	–

other hand, workers of *Veromessor pergandei, Pogonomyrmex badius, Manica* spp., and six of the seven *Myrmica* spp. readily followed artificial trails generated with extracts of their own poison glands. None of the species responded to extracts of either their Dufour's glands or their hind guts.

The results of the specificity studies are presented in Tables 1 and 2. Table 1 presents the results of intrageneric studies utilizing the seven *Myrmica* spp. Table 2 illustrates the responses of two *Myrmica* spp., three *Manica* spp., and *P. badius* workers to artificial trails treated with poison gland extracts derived from 15 myrmicine species in six genera.

With the exception of *M. monticola, Myrmica* spp. were almost equally sensitive to trail extracts of each other's poison glands (Table 1). *M. monticola* workers showed almost no propensity to follow their own poison gland extracts, whereas the other *Myrmica* spp. readily followed trails prepared from glandular extracts of this species. *Myrmica* workers readily followed these artificial trails and often circled them repeatedly. Trails prepared from *M. americana* glandular extracts were strongly active 1 hr after their preparation but were only weakly active after 3 hrs. Males and females of *M. emeryana* followed artificial trails as faithfully as workers of this species.

Poison gland extracts of the three *Manica* spp. did not appear to possess any specificity for the species in this genus (Table 2). The *Manica* spp. also followed artificial trails prepared from *Myrmica* poison glands as effectively

as they did their own, and two *Myrmica* spp. were equally responsive to extracts of *Manica* poison glands. As in the case of the *Myrmica* spp., *Manica* workers exhibited sustained trail following when exposed to these artificial trails. Furthermore, *Myrmica americana* and *M. brevinodis* readily followed poison gland extracts prepared from *Pogonomyrmex badius* and *P. barbatus* workers. Similarly, workers of *Manica bradleyi* exhibited trail following when exposed to trails containing extracts of these two species of *Pogonomyrmex* (Table 2). On the other hand, neither *Myrmica* nor *Manica* workers responded positively when bioassayed with poison gland extracts prepared from *Aphaenogaster fulva, Novomessor cockerelli*, and *Veromessor pergandei.*

Workers of *V. pergandei* were somewhat responsive (40 percent) to their own poison gland extracts but they seldom adhered to the circular trail for more than one complete traversal of its circumference. Similarly, workers of *P. badius,* which showed moderate trail-following activity in the presence of their own poison gland extracts as well as those derived from *Manica bradleyi* and *Myrmica brevinodis* (Table 2), exhibited very ephemeral trail following.

Workers of *T. septentrionalis, M. minimum, C. antarcticum, P. dentata, S. invicta*, and *C. lineolata* did not react to poison gland extracts of *M. brevinodis.*

DISCUSSION

At this juncture, the poison gland appears to be the primary source of odor trail pheromones in the Myrmicinae. The presence of chemical releasers of trail following in the venoms of *Myrmica, Manica*, and *Pogonomyrmex* species brings to 12 the number of myrmicine genera in which the trail pheromone has been localized in the poison gland secretion. Furthermore, since pheromonally-rich venoms have been identified in both primitive (*Myrmica*) and highly advanced (*Atta*) myrmicine genera, it seems clear that the utilization of the poison gland as a social organ is widespread in the Myrmicinae. On the other hand, Dufour's gland has been demonstrated to be the source of the trail pheromone in only two myrmicine genera, *Solenopsis* and *Pheidole* (Wilson, 1959, 1963). The utilization of the metathoracic tibial glands to generate chemical trails appears to be limited to the genus *Crematogaster* (Fletcher and Brand, 1968; Leuthold, 1968) and probably reflects an evolutionary specialization correlated with the apparent unsuitability of the gaster to function in trail laying.

Since the poison gland secretions of *Myrmica* and *Manica* species are almost totally lacking in specificity, it would appear that they may be utilizing the same or very similar trail pheromones, which would be consistent with the close relationship of these two myrmicine genera (Creighton, 1950). On the other hand, the genus *Pogonomyrmex* is certainly not closely related to *Myrmica* and *Manica* and the ability of workers in the latter two genera to follow artificial trails prepared from *Pogonomyrmex* poison gland extracts, and vice versa, may simply indicate that these three genera share common natural products in their

venoms. Indeed, other investigations demonstrate that phylgoenetic relationships are of little value in predicting the trail specificity of myrmicine poison gland secretions.

The poison gland secretion of the primitive ant *Daceton armigerum* (Latreille), a non-trail-laying myrmicine, releases strong trail following in attine species in three genera (Blum and Portocarrero, 1966). Similarly, the poison gland secretion of *Tetramorium guineense* (F.) is active as a trail pheromone for workers in two attine genera and vice versa. However, it is impossible to generalize about the specificity of the poison gland secretions of *Tetramorium* and the attines, since that of *T. caespitum* (L.) is not followed by workers of *T. guineense* or attine workers (Blum and Ross, 1965). Significantly, in transposition studies with poison gland secretions of *Monomorium* species, it has been established that the venom of one species releases strong trail following in another species but *not* vice versa (Blum, 1966). Thus, *M. minimum* workers will follow artificial trails prepared with *M. pharaonis* venom extracts as well as their own, but workers of *M. pharaonis* will not follow artificial *M. minimum* trails. Presumably, the venom of *M. pharaonis* contains the trail pheromone of *M. minimum,* but the latter species does not produce the trail pheromone of *M. pharaonis* in its poison gland secretion.

The trail pheromones derived from myrmicine venoms are certainly trace constituents which are not identified with the proteinaceous compounds which appear to dominate most of these secretions. The venoms of the *Manica, Myrmica,* and *Pogonomyrmex* species examined in this investigation are rich in proteins which are obviously not soluble in the solvent utilized to prepare active trail extracts. Tumlinson et al. (1971) have identified the major trail pheromone in the poison gland secretion of *Atta texana* (Buckley), methyl 4-methylpyrrole-2-carboxylate, as a trace constituent of a proteinaceous venom. Each ant is estimated to contain about 0.6 ng of this compound but four additional fractions are active in releasing trail following (Tumlinson et al., 1972). Possibly, these other trail pheromones may confer a degree of specificity which is unattainable with a single compound. It has also been suggested that the products of the Dufour's gland may be secreted in admixture with the poison gland secretion in order to obtain a more specific trail pheromone. Hölldobler and Wilson (1970) have demonstrated that *Pogonomyrmex badius* workers lay recruitment trails with the poison gland secretion, whereas the Dufour's gland products are utilized to set orientation marks. A combination of these two glandular exudates may produce a trail of much greater species specificity than could be obtained with either secretion alone.

Field studies with *M. brevispinosa* have demonstrated that trails are never laid by a foraging worker that is capable of bringing a food find back to the nest. Similarly, Eidmann (1927) noted that a worker of *M. rubra* did not lay a trail unless it was unable to transport the food to the nest by itself. Sig-

nificantly, *Myrmica* trails appear to be short lived both in the laboratory and in the field. In nature, the persistence of trails laid by myrmicine species varies greatly, which may be correlated with the recruitment strategies of the species as well as the vapor pressures of the trail pheromones. Thus, Ayre (1969) has demonstrated that workers of *M. americana*, after establishing a well-developed trail to a honey solution, quickly switch to topographical landmarks as a means of orienting between this food source and the nest. Since the trail is no longer being reinforced, it rapidly dissipates. Therefore, the relatively volatile trail pheromones which are characteristic of *Myrmica* species may be ideally suited to the foraging strategies employed by species in this genus. On the other hand, it would be selectively advantageous for those myrmicine species that do utilize the same trails daily to secrete trail pheromones which possess a relatively low vapor pressure. Indeed, artificial trails prepared from the poison gland secretion of *Atta texana* are highly active one week after their preparation (Blum et al., 1964). Members of this genus lay some of the most durable trails encountered in the Formicidae.

Among the species of *Myrmica*, *M. monticola* appears to be especially aberrant since it does not readily follow trails prepared from its own poison gland secretion or those of other *Myrmica* species. This species is also unusual because its mandibular gland chemistry is radically different from that of other *Myrmica* species which have been examined (Crewe and Blum, 1970). However, the singularity of *M. monticola* should serve to emphasize the variability in either behavior or natural product chemistry that may be encountered within the species in a genus. Ultimately, *M. monticola* may be demonstrated to be typical of many species which, because they lack some significant generic characters, are especially important as key indicators in the evolution of the genus.

Literature Cited

AYRE, G. L. 1969. Comparative studies on the behavior of three species of ants (Hymenoptera: Formicidae). II. Trail formation and group foraging. Can. Ent., **101**: 118–128.

BLUM, M. S. 1966. The source and specificity of trail pheromones in *Termitopone, Monomorium* and *Huberia* and their relation to those of some other ants. Proc. Roy. Ent. Soc. Lond. (A), **41**: 155–60.

BLUM, M. S., MOSER, J. C., AND CORDERO, A. D. 1964. Chemical releasers of social behavior. II. Source and specificity of the odor trail substances in four attine genera (Hymenoptera: Formicidae). Psyche, **71**: 1–7.

BLUM, M. S. AND PORTOCARRERO, C. A. 1966. Chemical releasers of social behavior. X. An attine trail substance in the venom of a non-trail laying myrmicine, *Daceton armigerum* (Latreille). Psyche, **73**: 150–155.

BLUM, M. S. AND ROSS, G. N. 1965. Chemical releasers of social behaviour. V. Source, specificity, and properties of the odour trail pheromone of *Tetramorium guineense* (F.). J. Insect Physiol., **11**: 857–868.

CREIGHTON, W. S. 1950. The ants of North America. Bull. Mus. Comp. Zool. Harvard, **104**: 1–585.

Crewe, R. M. and Blum, M. S. 1970. Alarm pheromones in the genus *Myrmica* (Hymenoptera: Formicidae). Z. Vergl. Physiol., **70:** 363–373.

Eidmann, H. 1927. Die Sprache der Ameisen. Rev. Zool. Russe, **7:** 39–47.

Fletcher, D. J. C. 1971. The glandular source and social functions of trail pheromones in two species of ants (*Leptogenys*). J. Ent. (A), **46:** 27–37.

Fletcher, D. J. C. and Brand, J. M. 1968. Source of the trail pheromone and method of trail laying in the ant *Crematogaster peringueyi*. J. Insect Physiol., **14:** 783–788.

Hölldobler, B. and Wilson, E. O. 1970. Recruitment trails in the harvester ant *Pogonomyrmex badius*. Psyche, **77:** 385–399.

Leuthold, R. H. 1968. A tibial gland scent-trail and trail-laying behavior in the ant *Crematogaster ashmeadi* Mayr. Psyche, **75:** 233–248.

Moser, J. C. and Blum, M. S. 1963. Trail marking substance of the Texas leaf-cutting ant: source and potency. Science, **140:** 1228.

Tumlinson, J. H., Moser, J. C., Silverstein, R. M., Brownlee, R. G., and Ruth, J. M. 1972. A volatile trail pheromone of the leaf-cutting ant *Atta texana*. J. Insect Physiol., **18:** 809–814.

Tumlinson, J. H., Silverstein, R. M., Moser, J. C., Brownlee, R. G., and Ruth, J. M. 1971. Identification of the trail pheromone of a leaf-cutting ant, *Atta texana*. Nature, **234:** 348–349.

Wilson, E. O. 1959. Source and possible nature of the odor trail of fire ants. Science, **129:** 643–644.

Wilson, E. O. 1963. The soil biology of ants. Ann. Rev. Ent., **8:** 345–368.